Attorney's Docket No.: 09010-017004 / DIVER 1240-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Patrick V. Warren et al. Art Unit : 1652
Serial No. : 09/481,733 Examiner : Elizabeth Slobodyansky, Ph.D.
Filed : January 11, 2000
Title : TRANSAMINASES AND AMINOTRANSFERASES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, David Weiner, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as a Principal Scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

3. I declare that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., screening enzymes, and nucleic acids encoding enzymes, for transaminase activity, was very high. Using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to express variants/ modifications of nucleic acids encoding the exemplary enzymes of the invention and screen them for expression of variant/ modified polypeptides having transaminase activity. One skilled in the art could have used routine protocols known in the art at the time of the invention, including those described in the instant specification, to screen for nucleic acids encoding polypeptides having 70% sequence identity to exemplary sequences of the invention, or active fragments thereof, for transaminase activity. One skilled in the art could have used routine protocols known in the art at the time of the invention, including

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I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

September 12 2003
Date of Deposit

/Norman Green/
Signature

Norman Green
Typed or Printed Name of Person Signing Certificate

those described in the instant specification, to screen for nucleic acids capable of hybridizing under the specific conditions set forth in the specification to exemplary sequences of the invention, or active fragments thereof, for transaminase activity. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (i.e., finding variant nucleic acids encoding transaminase) predictable. Furthermore, it would not have required any knowledge or guidance as to where modifications needed to be made to create variants/ modified polypeptides having transaminase activity. It would not have required any knowledge or guidance as to which are the specific structural elements, e.g., amino acid residues, that correlate with transaminase activity to create variants/ modifications of the exemplary nucleic acids and test them for the expression of polypeptides having transaminase activity. Accordingly, it would not have taken undue experimentation to make and use the claimed invention, including making and identifying of a genus of nucleic acids encoding transaminases and the enzymes they encode.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 9/11/03

David Weiner

David Paul Weiner, Ph.D.

Diversa Corporation
4955 Directors Place
San Diego, CA 92121
Phone: (858) 526 5228 Fax: (858) 526-5728
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Professional Experience

1997-present — Principal Scientist, Diversa Corporation.
- Discovery and evolution of novel enzymes
- Development of commercially important biocatalytic processes
- Design of efficient routes to important products by combining chemical and biological catalysis
- Director of multidisciplinary team of 15 scientists
- Proven track record in leading partner projects to success with large multinational corporations

1995-1997 — Postdoctoral Research Associate, Departments of Chemistry & Molecular Biology, The Scripps Research Institute. Advisor: Prof. Kim D. Janda.
- Designed and generated catalytic antibodies to hydrolyze nucleic acids and polypeptides.

1992-1995 — Postdoctoral Research Associate, Department of Chemistry, Amherst College. Advisor: Prof. David E. Hansen.
- Developed strategies for peptidolytic catalytic antibodies.

Education

1988-1992 — Ph.D. University of Leicester, U.K. Chemistry & Biochemistry
Thesis title: "Mechanistic studies on DNA gyrase from E. coli"
Thesis advisors: Prof. Paul M. Cullis & Prof. Anthony Maxwell

1985-1988 — B.Sc. (IIi honors) Biological Chemistry. University of Leicester, U.K.

1985 — 3 A-levels. Brooklands Technical College, U.K.

1982 — 12 O-levels. Kingston Grammar School, U.K.

Professional Societies

American Chemical Society
Royal Society of Chemistry
Faculty of 1000 (invited journal review panel)

Other Relevant Experience

Management skills: Certificate of Creative Leadership (CCL, San Diego).
Teamwork skills: Project leader on numerous projects. Mentor of several employees.
Language skills: Fluent Swedish, conversational French.

Grants Awarded

Single-cell screen for expression and activity (NIH SBIR phase I: 5/2001 to 11/2001). $119,840.

High throughput discovery of microbial epoxide hydrolases (NIH SBIR phase I: 2/2003 to 8/2003). $102,500 (Phase II submitted).

Directed Biosynthesis of Avermectin Derivatives (NIH SBIR phase I: 5/2003 to 10/2003). $90,300.

Publications

1. Dan E. Robertson, Jennifer A. Chaplin, Grace DeSantis, Mircea Podar, Mark Madden, Ellen Chi, Toby Richardson, Aileen Milan, Mark Miller, David P. Weiner, Kelvin Wong, Jeff McQuaid, Bob Farwell, Lori A. Preston, Xuqui Tan, Martin Keller, Eric Mathur, Patricia L. Kretz, Mark J. Burk, Jay M. Short. **A functional exploration of nitrilase protein sequence space.** (Submitted)

2. DeSantis, Grace; Zhu, Zuolin; Greenberg, William A.; Wong, Kelvin; Chaplin, Jenny; Hanson, Sarah R.; Farwell, Bob; Nicholson, Lawrence W.; Rand, Cynthia L.; Weiner, David P.; Robertson, Dan E.; Burk, Mark J. **An enzyme library approach to biocatalysis: development of nitrilases for enantioselective production of carboxylic acid derivatives.** Journal of the American Chemical Society (2002), 124(31), 9024-9025.

3. Brummer, Oliver; Gao, Changshou; Mao, Shenlan; Weiner, David P.; Janda, Kim D. **Design, synthesis and characterization of panning agents for the selection of metalloantibodies.** Letters in Peptide Science (1999), 6(5-6), 295-302.

4. Brummer, Oliver; Wentworth, Paul, Jr.; Weiner, David P.; Janda, Kim D. **Phosphorodithioates: synthesis and evaluation of new haptens for the generation of antibody acyl transferases.** Tetrahedron Letters (1999), 40(41), 7307-7310.

5. Weiner, David P.; Wiemann, Torsten; Wolfe, Mary M.; Wentworth, Paul, Jr.; Janda, Kim D. **A Pentacoordinate Oxorhenium(V) Metallochelate Elicits Antibody Catalysts for Phosphodiester Cleavage.** Journal of the American Chemical Society (1997), 119(17), 4088-4089.

6. Cullis, Paul M.; Maxwell, Anthony; Weiner, David P. **Exploiting Nucleotide Thiophosphates To Probe Mechanistic Aspects of Escherichia coli DNA-Gyrase.** Biochemistry (1997), 36(20), 6059-6068.

7. Smith, Robert M.; Weiner, David P.; Chaturvedi, Nishith C.; Thimblin, Michael D., Jr.; Raymond, Susan J.; Hansen, David E. **Norbornyl dipeptide analogs: mimics of both a transition state and a torsionally distorted ground state.** Bioorganic Chemistry (1995), 23(4), 397-414.

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9. Cullis, Paul M.; Maxwell, Anthony; Weiner, David P. **Energy coupling in DNA gyrase: a thermodynamic limit to the extent of DNA supercoiling.** Biochemistry (1992), 31(40), 9642-6.

10. Weiner, David P. **Expanding the chemistry of nature: catalytic antibodies.** Genetic Engineer and Biotechnologist (1992), 12(2), 9-13.

11. Weiner, David P. **Catalytic antibodies.** Chemistry & Industry (London, United Kingdom) (1991), (10), 347-9.

12. Modha, Jay; Weiner, David P.; Cullis, Paul M.; Rivett, Jennifer. **Effects of ATP analogs on the activity of the lon proteinase of Escherichia coli.** Biochemical Society Transactions (1990), 18(4), 589.

Patents

1. Greenberg, William; Weiner, David Paul; Adger, Brian; Burk, Mark. **Biocatalytic reduction of nitro groups.** PCT Int. Appl. (2003), 63pp., (WO 0362399 A2 20030731 AN 2003:591312)

2. Barton, Nelson R.; Weiner, David Paul; Greenberg, William; Luu, Samantha; Chang, Kristine; Waters, Elizabeth. **Amidases, nucleic acids encoding them, and methods for making and using them.** PCT Int. Appl. (2003), 204 pp., (WO 0364613 A2 20030807 AN 2003:610600)

3. Desantis, Grace; Chaplin, Jennifer Ann; Chi, Ellen; Milan, Aileen; Short, Jay M.; Weiner, David; Maddes, Mark; Madden, Darcy; Burk, Mark J.; Mathur, Eric. **Environmental microbe nitrilase and gene sequences exhibiting stereoselectivity useful for synthesis of chiral reaction products.** U.S. Pat. Appl. (2003), 105 pp., (US 20030124698 A1 20030703 AN 2003:511968)

4. Weiner, David; Burk, Mark; Hitchman, Tim; Pujol, Catherine; Richardson, Toby; Short, Jay. **Screening, selection, identification and sequences of cytochrome P 450 for use in the production of chiral epoxides.** PCT Int. Appl. (2003), 365 pp. (WO 0352050 A2 20030626 AN 2003:491367)

5. Stege, Justin; Preston, Lori; Weiner, David. **Fusion proteins comprising enzyme, intein, and detectable moiety domains and their use in methods for normalizing enzymic assays.** PCT Int. Appl. (2003), 103 pp. (WO 0350265 A2 20030619 CAN 139:49103 AN 2003:472619)

6. Zhao, Lishan; Mathur, Eric; Weiner, David; Richardson, Toby; Milan, Aileen; Burk, Mark; Han, Bin. **Identification, cloning and sequences of epoxide hydrolases and their use for enantiomeric hydrolysis of epoxides and arene oxides.** PCT Int. Appl. (2003), 400 pp. (WO 0312126 A2 20030213 CAN 138:165742 AN 2003:118029)

7. Weiner, David; Hitchman, Tim; Zhao, Lishan; Burk, Mark. **Methods for the manufacture of pure single enantiomer compounds and for selecting enantioselective enzymes.** PCT Int. Appl. (2003), 122 pp. (WO 0300909 A2 20030103 CAN 138:54642 AN 2003:6148)

8. Madden, Mark; Desantis, Grace; Chaplin, Jennifer Ann; Weiner, David Paul; Milan, Aileen; Chi, Ellen; Short, Jay M.; Burk, Mark. **Bacterial nitrilase and gene sequences exhibiting stereoselectivity useful for synthesis of chiral reaction products.** PCT Int. Appl. (2003), 560 pp. (WO 0300840 A2 20030103 CAN 138:68923 AN 2003:6085)

9. Madden, Mark; Weiner, David Paul; Chaplin, Jennifer Ann. **Producing enantiomerically pure α-substituted carboxylic acids using stereospecific nitrilases in the presence of**

Strecker reagents. PCT Int. Appl. (2001), 87 pp. (WO 0148175 A2 20010705 CAN 135:89140 AN 2001:489604)

Selected Conference Presentations

Invited Speaker at the Materials Research Outreach Symposium, January 29-31, 2003, University of Santa Barbara, CA. Talk title: "New opportunities in Biocatalysis".

Keynote speaker at the 2002 DOE Catalysis and Chemical Transformations Workshop, September 20-22, 2002, Chicago, IL. Talk title: "New opportunities in Biocatalysis".

Invited speaker at the 9th International Symposium on the Genetics of Industrial Microorganisms, July 1-5,2002, Gyeongju, Korea. Talk title: "Discovering and Evolving the Best Genes From Nature".

Invited speaker at the Development Center for Biotechnology, July 15,2002, Taipei, Taiwan. "Discovering and Evolving the Best Genes From Nature".

Invited speaker at the Local Chapter of the American Chemical Society, San Diego, November 13, 2001. "Discovery and Evolution of Novel Biocatalysts".

Speaker at the American Oil Chemists Society, July 20, 2000, San Diego. "Discovery and Evolution of Novel Enzymes"

Biocatalysis Gordon Research Conference, Proctor Academy, Andover, NH, July 1998. "Novel enzymes from biodiversity".

Presentations at Companies

Over 100 presentations on Diversa technologies at multinational companies world-wide.

EXHIBIT A

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which comprises SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

EXHIBIT B

United States Patent **6,596,926**
Famodu , et al. **July 22, 2003**

Phosphatidylcholine biosynthetic enzymes

Abstract

This invention relates to an isolated nucleic acid fragment encoding phosphatidylethanolamine N-methyltransferase biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the phosphatidylethanolamine N-methyltransferase biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of phosphatidylethanolamine N-methyltransferase biosynthetic enzyme in a transformed host cell.

Inventors: **Famodu; Omolayo O.** (Newark, DE); **Kinney; Anthony J.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **668262**

Filed: **September 22, 2000**

Current U.S. Class: **800/281**; 435/6; 435/69.1; 435/183; 435/410; 435/419; 435/252.3; 435/320.1; 530/350; 530/370; 536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.33; 800/278; 800/295

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N 005/14; C12N 009/00

Field of Search: 435/6,69.1,183,410,419,252.3,320.1 530/350,370 536/23.2,23.6,24.1,24.3,24.33 800/278,295,281

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Xiaoying Lin et al., Nature, vol. 402:761-768, Dec. 16, 1999, Sequence and Analysis of Chromosome 2 of the plant Arabidopsis thaliana.
Patricia McGraw et al., Genetics, vol. 122:317-330, Jun. 1989, Mutations in the Saccharomyces cerevisiae opi3 Gene: Effects on Phospholipid Methylation, Growth and Cross-Pathway Regulation of Inositiol Synthesis.

USPN 6,596,926

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Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/155,626, filed Sep. 23, 1999.

Claims

What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having phosphatidylethanolamine N-methyltransferase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 80% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 85% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 90% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 95% sequence ***identity*** based on the Clustal alignment method.

5. The ***polynucleotide*** of claim 1 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:20.

6. The ***polynucleotide*** of claim 1 wherein the nucleotide sequence comprises the

nucleotide sequence of SEQ ID NO:19.

7. A vector comprising the ***polynucleotide*** of claim 1.

8. A recombinant DNA construct comprising the ***polynucleotide*** of claim 1 operably linked to a regulatory sequence.

9. A method for transforming a cell comprising transforming a cell with the ***polynucleotide*** of claim 1.

10. A cell comprising the recombinant DNA construct of claim 8.

11. A method for producing a plant comprising transforming a plant cell with the ***polynucleotide*** of claim 1 and regenerating a plant from the transformed plant cell.

12. A plant comprising the recombinant DNA construct of claim 8.

13. A seed comprising the recombinant DNA construct of claim 8.

United States Patent **6,593,514**
Cahoon , et al. **July 15, 2003**

Method for the production of calendic acid, a fatty acid containing delta-8,10,12 conjugated double bonds and related fatty acids having a modification at the delta-9 position

Abstract

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with modification of the delta-9 position of fatty acids, in particular, formation of conjugated double bonds are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles. The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with formation of a trans delta-12 double bond also are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

Inventors: **Cahoon; Edgar Benjamin** (Wilmington, DE); **Hitz; William Dean** (Wilmington, DE); **Ripp; Kevin G.** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **638937**

Filed: **August 15, 2000**

Current U.S. Class: **800/281**; 800/298; 435/69.1; 435/419; 536/23.6

Intern'l Class: A01H 005/00; C12N 015/82; C07H 021/04

Field of Search: 800/281,298 435/69.1,419 536/23.6

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5231020	Jul., 1993	Jorgensen et al.
5428072	Jun., 1995	Cook et al.
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Joan T. Odell et al., Nature, vol. 313:810-812, 1985, Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S Promoter.
Mark D. Adams et al., Science, vol. 252:1651-1656, 1991, Complementary DNA Sequencing: Expressed Sequence Tags and Human Genome Project.
M. D. Chisholm et al., Can. Journ. of Biochem., vol. 42:1033-1040, 1964, Biosynthesis of Mustard Oil Glucosides.
R. C. Thiel et al., J. Anim. Sci., vol. 77(suppl):47, 1998, Effects of CLA supplementation on quality and sensory characteristics of pork.
R. C. Wiegand et al., J. Anim. Sci., vol. 77(suppl):47, 1999, Effects of CLA supplementation on pork quality characteristics in crossbred growing-finishing barrows.
Linda Gritz et al., Gene, vol. 25: 179-188, 1983, Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in Escherichia coli and Saccharomyces cerevisiae.
Mats Hamberg et al., Biochem. & Biophys. Res. Comm., vol. 188(3):1992, Metabolism of 6,9,12-Octadecatrienoic acid in the red alga lithothamnion corallioides: Mechanism of formation of a conjugated tetraene fatty acid.
Edgar B. Cahoon et al., J. Biol. Chem., vol. Manuscript Moo9188200, No. in press, 2000, pp. 1-27, Formation of conjugated delta 8, delta 10 double bonds by delta 12-oleic acid desaturase related enzymes: Biosynthetic origin of calendic acid.
Kathrin Fritsche et al., FEBS Lett., vol. 462:249-253, 1999, Isolation and characterization of a calendic acid producing (8,11)-linoleoyl desaturase.

Primary Examiner: McElwain; Elizabeth F.

Parent Case Text

This application claims priority benefit of U.S. Provisional Application No. 60/149,050 filed Aug. 16, 1999, now abandoned.

Claims

What is claimed is:

1. A chimeric gene comprising an isolated ***nucleic acid*** fragment encoding a plant fatty acid modifying enzyme associated with conjugated double bond formation comprising a delta-9 position of fatty acids having an amino acid ***identity*** of at least 72.5% based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2 or 4

wherein said fragment or a functionally equivalent subfragment thereof or a complement thereof is operably linked to suitable regulatory sequences.

2. The chimeric gene of claim 1 wherein the ***nucleic acid*** fragment is isolated from Calendula officinalis.

3. The chimeric gene of claim 1 wherein the plant fatty acid modifying enzyme is associated with the formation of calendic acid.

4. A transformed host cell or plant comprising in its genome the chimeric gene of claim 1.

5. A transformed host cell or plant comprising in its genome the chimeric gene of claim 2.

6. A transformed host cell or plant comprising in its genome the chimeric gene of claim 3.

7. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 1;

(b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

8. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 2;

(b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

9. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 3;

(b) growing the transformed host cell or plant under conditions suitable for the expression

of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

10. The method of claim 7, 8, or 9 wherein the host cell or plant is selected from the group consisting of plant cells and microorganisms.

11. The method of claim 7, 8, or 9 and wherein the level of calendic acid is altered.

12. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 1;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

13. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 2;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

14. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 3;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

15. The method of claim 12, 13, or 14 wherein the fatty acid modifying enzyme is associated with the formation of calendic acid or dimorphecolic acid.

United States Patent 6,590,141
Frohberg July 8, 2003

Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

Abstract

Nucleic acid molecules are described which encode enzymes which participate in starch synthesis in plants. These enzymes are a new isoform of starch synthase. There are furthermore described vectors for generating transgenic plant cells and plants which synthesize a modified starch. There are furthermore described methods for the generation of these transgenic plant cells and plants, and methods for producing modified starches.

Inventors: **Frohberg; Claus** (Berlin, DE)
Assignee: **Aventis CropScience GmbH** (Frankfurt, DE)
Appl. No.: **638524**
Filed: **August 11, 2000**

Foreign Application Priority Data

Aug 11, 1999[DE] 199 37 348

Current U.S. Class: 800/284; 800/278; 800/286; 800/320.1; 435/69.1; 435/101; 435/320.1; 435/419; 435/468; 536/23.6
Intern'l Class: C12N 015/29; C12N 015/82; C12N 005/04; A01H 005/00; C12P 019/04
Field of Search: 536/23.6 435/69.1,468,320.1,419,101 800/278,284,320.1,286

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WO 99/24575	May., 1999	WO.

Other References

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Kossmann et al. Progress Biotechnol. 10:271-278, 1995.*
Sasaki, T. Accession No. C99345, 1996.*
Database Accession No. C99345 published Oct. 16, 1998, also referred to as XP 002163786.
Database Accession No. AI94842 published Aug. 23, 1999, also referred to as XP 002163787.
Cao, Heping et al, "Identification of the Soluble Starch Synthase Activities of Maize Endosperm", Plant Physiology, May 1999, vol. 120, pp. 205-215, No. 1.

Primary Examiner: Fox; David T.
Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

Claims

I claim:

1. An isolated ***nucleic acid*** molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of

(a) ***nucleic acid*** molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;

(b) ***nucleic acid*** molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;

(c) ***nucleic acid*** molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;

(d) ***nucleic acid*** molecules whose nucleotide sequence deviates from the sequence of the ***nucleic acid*** molecules mentioned under (a), (b) or (c) owing to the degeneracy of the genetic code;

(e) ***nucleic acid*** molecules which have over 85% sequence ***identity*** with SEQ ID NO:1; and

(f) ***nucleic acid*** molecules which constitute allelic variants of the nucleic acid molecules indicated under (a), (b), (c), (d) or (e).

2. The ***nucleic acid*** molecule as claimed in claim 1 which is a DNA molecule.

3. The ***nucleic acid*** molecule as claimed in claim 1 which is an RNA molecule.

4. A vector comprising a ***nucleic acid*** molecule as claimed in claim 1.

5. The vector as claimed in claim 4 comprising one or more regulatory elements which ensure the transcription of said ***nucleic acid*** molecules and/or the synthesis of a translatable RNA in a pro- and/or eukaryotic cell.

6. The vector as claimed in claim 4, wherein said ***nucleic acid*** molecule is linked in sense orientation to regulatory elements which ensure the transcription and synthesis of a translatable RNA in pro- and/or eukaryotic cells, or wherein said ***nucleic acid*** molecule is linked in anti-sense orientation to regulatory elements which ensure the transcription and synthesis of a non-translatable RNA in pro- and/or eukaryotic cells.

7. A host cell which is transformed with a ***nucleic acid*** molecule as claimed in claim 1 or a vector as claimed in claim 4, or a cell which is derived from the host cell and which comprises the vector of claim 4.

8. The host cell as claimed in claim 7 which is a plant cell.

9. A method for producing a protein encoded by the ***nucleic acid*** molecule of claim 1, in which a host cell as claimed in claim 7 is cultured under conditions which permit the synthesis of the protein, and the protein is isolated from the cultured cells and/or the culture medium.

10. The plant cell of claim 8, wherein said ***nucleic acid*** molecule which encodes a protein with the bioactivity of a starch synthase is under the control of regulatory elements which permit the transcription of a translatable mRNA in plant cells.

11. The plant cell of claim 8, wherein the activity of a protein encoded by the ***nucleic acid*** molecule of claim 1 is increased in this plant cell compared with corresponding, non-genetically-modified plant cells from wild-type plants.

12. A plant comprising plant cells as claimed in claim 8.

13. The plant as claimed in claim 12 which is a crop plant.

14. The plant as claimed in claim 12 which is a starch-storing plant.

15. The plant as claimed in claim 12 which is a maize plant.

16. A method for generating a transgenic plant cell, wherein a plant cell is subjected to genetic modification by introducing a ***nucleic acid*** molecule as claimed in claim 1 and/or a vector as claimed in claim 4.

17. A method for generating a transgenic plant, wherein

(a) a plant cell is subjected to genetic modification by introducing a ***nucleic acid*** molecule as claimed in claim 1 and/or a vector as claimed in claim 4; and

(b) a plant is regenerated from this cell; and, if appropriate,

(c) more plants are generated from the plant of (b).

18. Propagation material of a plant comprising plant cells as claimed in claim 8.

19. A method for producing a modified starch obtained from the host cell of claim 8, from the plant of claim 12, or from the propagation material of 18, comprising the step of extracting the starch from a plant cell as claimed in claim 8, from a plant as claimed in claim 12 and/or from propagation material as claimed in claim 18.

United States Patent **6,586,215**
Yaver , et al. **July 1, 2003**

Polypeptides having peroxidase activity and nucleic acids encoding same

Abstract

The present invention relates to isolated polypeptides having peroxidase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

Inventors: **Yaver; Debbie** (Davis, CA); **McArdle; Barbara** (Davis, CA)
Assignee: **Novozymes Biotech, Inc.** (Davis, CA)

Appl. No.: **885329**
Filed: **June 19, 2001**

Current U.S. Class:	435/192; 435/6; 435/320.1; 435/325; 435/252.3; 536/23.1; 536/23.2
Intern'l Class:	C12N 009/08; C12N 015/00; C12N 005/00; C12Q 001/68; C07H 021/04
Field of Search:	435/192,6,252.3,320.1 536/23.2,23.1

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Primary Examiner: Monshipouri; M.
Attorney, Agent or Firm: Stames; Robert L.

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 09/596,824 filed Jun. 19, 2000 now U.S. Pat. No. 6,372,464 issued Apr. 16, 2002, which application is fully incorporated herein by reference.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** sequence encoding a polypeptide having peroxidase activity, selected from the group consisting of:

(a) a ***nucleic acid*** sequence encoding a polypeptide having an amino acid sequence which has at least 75% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6, or at least 85% ***identity*** with amino acids 22 to 385 of SEQ ID NO:4;

(b) a ***nucleic acid*** sequence encoding a polypeptide having an amino acid sequence which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5, or at least 85% homology with nucleotides 2008 to 3462 of SEQ ID NO:3;

(c) a ***nucleic acid*** sequence which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii); and

(d) a fragment of (a), (b), or (c), which encodes a polypeptide having peroxidase activity.

2. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide having an amino acid sequence which has at least 75% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

3. The ***nucleic acid*** sequence of claim 2, which encodes a polypeptide having an amino acid sequence which has at least 80% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

4. The ***nucleic acid*** sequence of claim 3, which encodes a polypeptide of having an amino acid sequence which has at least 85% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

5. The ***nucleic acid*** sequence of claim 4, which encodes a polypeptide having an amino acid sequence which has at least 90% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

6. The ***nucleic acid*** sequence of claim 5, which encodes a polypeptide having an amino

acid sequence which has at least 95% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6 .

7. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6 .

8. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or a fragment thereof having peroxidase activity.

9. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO6.

10. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide which consists of amino acids 22 to 370 of SEQ ID NO:2, amino acids 22 to 365 of SEQ ID NO:4, or amino acids 19 to 362 of SEQ ID NO:6.

11. The ***nucleic acid*** sequence of claim 1, which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

12. The ***nucleic acid*** sequence of claim 11, which has at least 80% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

13. The ***nucleic acid*** sequence of claim 12, which has at least 85% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

14. The ***nucleic acid*** sequence of claim 13, which has at least 90% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

15. The ***nucleic acid*** sequence of claim 14, which has at least 95% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

16. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:1.

17. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5.

18. The ***nucleic acid*** sequence of claim 1, which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) me cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO;1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2845 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii).

19. The ***nucleic acid*** sequence of claim 1, which is contained in plasmid pBM37-7 which is contained in E. coli NRRL B-30280, plasmid pBM38-1 which is contained in E. coli NRRL B-30281, or plasmid pBM39-1 which is contained in E. coli NRRL B-30282.

20. A ***nucleic acid*** construct comprising the ***nucleic acid*** sequence of claim 1, operably linked to one or more control sequences which direct the production of the polypeptide in a suitable expression host.

21. A recombinant expression vector comprising the ***nucleic acid*** construct of claim 20, a promoter, and transcriptional and translational stop signals.

22. A recombinant host cell comprising the ***nucleic acid*** construct of claim 20.

23. A method for producing a polypeptide having peroxidase activity comprising

(a) cultivating the host cell of claim 22, under conditions suitable for production of the polypeptide; and

(b) recovering the polypeptide.

United States Patent **6,586,179**
Jegla , et al. **July 1, 2003**

Human Eag2

Abstract

The invention provides isolated nucleic acid and amino acid sequences of Eag2, antibodies to Eag2, methods of detecting Eag2, and methods of screening for modulators of Eag2 potassium channels using biologically active Eag2. The invention further provides, in a computer system, a method of screening for mutations of human Eag2 genes as well as a method for identifying a three-dimensional structure of Eag2 polypeptide monomers.

Inventors: **Jegla; Timothy J.** (Durham, NC); **Liu; Yi** (Cary, NC)
Assignee: **ICAgen, Incorporated** (Durham, NC)

Appl. No.: **614480**
Filed: **July 10, 2000**

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Intern'l Class:	C12Q 001/68; C07H 017/00; C12P 021/06; C07K 014/00
Field of Search:	536/23.1 435/7.1,325,320.1,252.3 530/350 436/6

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Primary Examiner: Carlson; Karen Cochrane
Attorney, Agent or Firm: Townsend and Townsend and Crew LLP

Parent Case Text

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 60/143,467, filed Jul. 13, 1999, herein incorporated by reference in its entirety.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and wherein said ***nucleic acid*** specifically hybridizes under stringent conditions to SEQ ID NO:1, wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

2. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and

(ii) comprises an amino acid sequence that has greater than 85% amino acid ***identity*** to the amino acid sequence of SEQ ID NO:2.

3. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide specifically binds to polyclonal antibodies generated against SEQ ID NO:2.

4. The isolated ***nucleic acid*** of claim 1, wherein the ***nucleic acid*** encodes human Eag2.

5. The isolated acid of claim 1, wherein the ***nucleic acid*** encodes an amino acid sequence of SEQ ID NO:2.

6. The isolated ***nucleic acid*** sequence of claim 1, wherein the ***nucleic acid*** has a nucleotide sequence of SEQ ID NO:1.

7. The isolated ***nucleic acid*** of claim 1, wherein the ***nucleic acid*** is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as primers selected from the group consisting of:

ATGCCGGGGGGCAAGAGAGGGCTG (SEQ ID NO:3);

CTGACCCTAAGCTCATAAGGATGAAC (SEQ ID NO:4);

CCACCTCATCATCCTGGATGACTTCC (SEQ ID NO:5);

TTAAAAGTGGATTTCATCTTTGTCAGATTCAGG (SEQ ID NO :6);

GGGGACCTCATTTACCATGCTGGAG (SEQ ID NO:7);

GATTCCCTCATCCACATTTTCAAAGGC (SEQ ID NO:8);

and wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

8. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a homomeric channel.

9. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a heteromeric channel.

10. An expression vector comprising the ***nucleic acid*** of claim 1.

11. A host cell transfected with the vector of claim 10.

12. A method of detecting a ***nucleic acid***, the method comprising contacting a sample comprising a first ***nucleic acid*** with an isolated second nucleic acid of claim 1 and

detecting hybridization of the second ***nucleic acid*** to the first ***nucleic acid***, thereby detecting the first ***nucleic acid***.

United States Patent 6,583,337
Allen , et al. **June 24, 2003**

Plant glucose-6-phosphate translocator

Abstract

This invention relates to an isolated nucleic acid fragment encoding a glucose-6-phosphate/phosphate translocator. The invention also relates to the construction of a chimeric gene encoding all or a portion of the glucose-6-phosphate/phosphate translocator, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the glucose-6-phosphate/phosphate translocator in a transformed host cell.

Inventors: **Allen; Stephen M.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **436521**

Filed: **November 9, 1999**

Current U.S. Class: **800/278**; 435/6; 435/69.1; 435/71.1; 435/183; 435/410; 435/419; 435/418; 435/252.3; 435/320.1; 530/350; 530/370; 536/23.1; 536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.5

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N 005/14; C12N 009/00

Field of Search: 435/6,69.1,71.1,183,410,419,418,252.3,320.1 530/370,350 536/23.1,23.2,23.6,24.1,24.3,24.5

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NCBI General Identifier No. 2997591.
NCBI General Identifier No. 2997589.

Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims priority benefit to U.S. Provisional Application No. 60/107,910 filed Nov. 10, 1998, now abandoned.

Claims

What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having glucose-6-phosphate/phosphate translocator activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 86% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:3.

5. The ***polynucleotide*** of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:4.

6. A recombinant DNA construct comprising the ***polynucleotide*** of claim 1 operably linked to a regulatory sequence.

7. A method for transforming a cell comprising transforming a cell with the ***polynucleotide*** of claim 1.

8. A cell comprising the recombinant DNA construct of claim 6.

9. A method for producing a plant comprising transforming a plant cell with the ***polynucleotide*** of claim 1 and regenerating a plant from the transformed plant cell.

10. A plant comprising the recombinant DNA construct of claim 1.

11. A seed comprising the recombinant DNA construct of claim 1.

12. A vector comprising the ***polynucleotide*** of claim 1.

United States Patent **6,582,950**
Smith , et al. **June 24, 2003**

C3 binding polypeptide of Streptococcus agalactiae group b Streptococcus

Abstract

This invention relates to the identification of a human complement C3 binding polypeptide and the nucleic acid which encodes the polypeptide from Streptococcus agalactiae. The polypeptide binds C3 and may be implicated in S. agalactiae adhesion and/or virulence. The polypeptide is conserved in mass in a variety of streptococcal isolates and is recognized by antibodies produced by humans exposed to or colonized with Group B Streptococcus.

Inventors: **Smith; Beverly L.** (Minneapolis, MN); **Ferrieri; Patricia** (Minneapolis, MN)

Assignee: **Regents of the University of Minnesota** (Minneapolis, MN)

Appl. No.: **610199**

Filed: **July 1, 2000**

Current U.S. Class: 435/252.3; 435/320.1; 435/325; 536/23.7

Intern'l Class: C12N 001/20; C12N 015/00; C12N 005/00; C07H 021/04

Field of Search: 424/190.1 435/69.3,252.33,253.4,252.3,320.1,325 536/23.7

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Primary Examiner: Navarro; Mark
Attorney, Agent or Firm: Mueting Raasch & Gebhardt

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. .sctn.119(e) of U.S. Provisional Patent Application No. 60/157,550, filed on Oct. 4, 1999, and U.S. Provisional Patent Application No. 60/173,766, filed on Dec. 30, 1999, both of which are hereby incorporated by reference.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** fragment that hybridizes to at least a portion of at least one of the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their

complementary strands under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0.1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., said isolated ***nucleic acid*** fragment encodes a polypeptide that binds human complement C3 protein.

2. The ***nucleic acid*** fragment of claim 1 isolated from S. agalactiae.

3. The ***nucleic acid*** fragment of claim 1 which encodes a polypeptide represented by. SEQ ID NO:5.

4. The ***nucleic acid*** fragment of claim 1 in a ***nucleic acid*** vector.

5. The ***nucleic acid*** fragment of claim 4 wherein the ***nucleic acid*** vector is an expression vector capable of producing a polypeptide.

6. An isolated ***nucleic acid*** having at least 50% ***nucleic acid identity*** to the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4, and which hybridizes under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0. 1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., to at least a portion of at least one of the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands, said ***nucleic acid*** encoding a polypeptide that binds human complement C3 protein.

7. An isolated ***polynucleotide*** encoding a polypeptide comprising the amino acids represented by SEQ ID NO:5.

8. The ***polynucleotide*** of claim 7 wherein the polypeptide binds human complement C3.

9. An isolated host cell comprising a ***nucleic acid*** fragment of claim 1.

10. The cell of claim 9 wherein the cell is a bacterium or a eukaryotic cell.

11. An isolated ***nucleic acid*** fragment comprising SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands.

12. An isolated RNA transcribed from a double-stranded ***nucleic acid*** comprising a ***nucleic acid*** fragment of claim 2.

13. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 50% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

14. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 60% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

15. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 70% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

16. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 80% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

17. An isolated ***nucleic acid*** fragment consisting essentially of at least 30 nucleotides of SEQ ID NO:4, wherein said ***nucleic acid*** fragment encodes a polypeptide that binds to human complement C3 protein.

United States Patent **6,541,684**
Bowen , et al. **April 1, 2003**

Nucleotide sequences encoding maize RAD51

Abstract

Nucleic acid sequences encoding two RAD51 recombinases active in maize plants are provided. cDNA sequences including the ZmRAD51 coding sequences and unique 3'-untranslated regions which are useful as RFLP probes, are also provided. The production of plasmids containing a nucleic acid sequence encoding a ZmRAD51 fusion protein, as well as the use of the plasmids to introduce the ZmRAD51 coding sequence into a host cell, such as maize cell, are also disclosed.

Inventors: **Bowen; Benjamin A.** (Hayward, CA); **Chamberlin; Mark A.** (Windsor Heights, IA); **Drummond; Bruce J.** (Des Moines, IA); **McElver; John A.** (Durham, NC); **Rothstein; Rodney J.** (Maplewood, NJ)

Assignee: **Trustees of Columbia University in the City of New York** (New York, NY); **Pioneer Hi-Bred International, Inc.** (Johnston, IA)

Appl. No.: **246963**

Filed: **February 9, 1999**

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Intern'l Class: A01H 005/00

Field of Search: 536/23.1,23.5,24.1 435/410,468,196,69.1 800/298,320.1

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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/074,745, filed Feb. 13, 1998 and is herein incorporated by reference.

Claims

What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

a) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 7;

b) a polynucleotide having at least 90% identity to a polynucleotide of (a);

c) a polynucleotide which will hybridize under ***stringent hybridization*** conditions to said polynucleotide of (a) or (b); and

d) a polynucleotide comprising at least 30 contiguous nucleotides from a polynucleotide of (a), (b) or (c);

wherein the polynucleotide of (a), (b) or (c) encodes a polypeptide with recombinase activity.

2. The isolated polynucleotide of claim 1, wherein said polynucleotide has a sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 6.

3. An expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter.

4. The host cell transfected with an expression cassette of claim 3.

5. The host cell of claim 4, wherein said host cell is a bacterial cell.

6. The host cell of claim 4, wherein said host cell is a sorghum or maize cell.

7. A method of making maize recombinase comprising the steps of:

a) transforming or transfecting a host cell with the expression cassette of claim 3; and

b) purifying the recombinase from the host cell.

8. The method of claim 7, wherein the host cell is selected from the group consisting of a bacterial cell, a plant cell, a mammalian cell and a yeast cell.

9. A method of modulating ZmRAD 51 activity in a plant, comprising:

(a) introducing into a plant cell an expression cassette comprising an isolated polynucleotide of claim 1 operatively linked to a promoter;

(b) culturing the plant cell under plant cell growing conditions;

(c) regenerating a plant which possesses the transformed genotype, and

(d) inducing expression of said polynucleotide for a time sufficient to modulate ZmRAD51 activity in said plant.

10. A transgenic plant cell comprising an isolated polynucleotide of claim 1.

11. A transgenic plant comprising an isolated polynucleotide of claim 1.

12. A transgenic seed from the transgenic plant of claim 11.

13. Primer pairs for isolating at least a part of a Zea mays recombinase gene, selected from the group consisting of SEQ ID NOS: 12 and 13, SEQ ID NOS: 14 and 19, SEQ IDS NOS: 14 and 20, and SEQ ID NOS: 14 and 15, or complements thereof.

14. An RFLP probe for a maize recombinase gene comprising at least 30 nucleotides residues of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.